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MICROSPORIDIA PARASITIC IN COPEPODS *

R. Kudo

While working on Cnidosporidia of various aquatic animals at Spring Valley, New York, during the summer of 1920, my attention was called to microsporidian infections in *Cyclops albidus* and *Cyclops fuscus.* Notwithstanding the fact that an enormous number of papers dealing with the occurrence, taxonomy and biology of several species of copepods have been published, it is strange to find but a few brief notes recording the occurrence of microsporidia-like parasites in the animals under consideration. Aside from one brief paper by Schröder (1914), all the others, which will be reviewed later, were published in Europe between 1887 and 1895 and the microsporidian nature of the forms described, is open to question. I have failed to find any record from any other land than Europe. In view of the circumstances, it seems worth while to describe the microsporidia which have come under my observation, and to review the old European records.

Although six species, i. e., *Cyclops albidus, C. fuscus, C. ater, C. phaleratus, C. prasinus* and *C. serrulatus,* were examined, only the first two species were found to be infected by the microsporidian parasites. In each collection, the number of individuals of *Cyclops albidus* predominated over the other five species. The absence of the infected animals in the latter four species may be due to the small number of individuals examined, and not to the specific difference in the host. It seemed to me that there could not be any special factor or factors that might have caused the infection to occur among the first two host species.

Before the examination for the microsporidian parasites, each host animal was placed on a slide, and identified by means of Marsh's key (1918). The animal was then slightly pressed under a cover glass, and examined microscopically in either fresh and stained smears or section preparations. The methods of fixation and staining, and also of the extrusion of the polar filament were exactly same to those which I have used in my previous studies (Kudo, 1920, 1920a).

Nosema cyclopis nov. spec.

Habitat: In the fat bodies and reproductive organs (?) of *Cyclops fuscus.* Spring Valley, New York (August).

Two, one male and the other female, out of twenty-two individuals collected from a creek on August 20, were found to harbor the Microsporidian. Six fixed specimens from Conger's Lake and twenty-four from the outlet of the latter were free from the infection.

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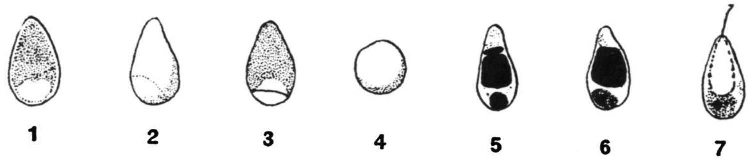
The two infected animals were strikingly whitish opaque in color compared with the normal ones. The host did not suffer any decrease of the activity. This is probably due to the fact that the parasites do not attack the muscle cells. Concerning the effect of the parasitism upon the host body, I cannot make any definite statement as the number of the infected animals were small and they were not kept alive for any length of time under observation. However, there seems to be little doubt as to the fatal outcome of the infection.

The spores are pyriform (Figs. 1 to 7). The anterior end is rounded at its tip, while the posterior margin is broadly rounded. The spore membrane is very thin. The spore is less refractive than that of any species which I have studied up to the present. In cross-section, the spore is circular (Fig. 4). The broadest portion of the spore is located near the posterior extremity. The shape and size are constant. In the fresh state, there is always seen an oval clear space at the posterior end of the spore (Figs. 1 to 3). The rest of the cavity of the spore is filled with finely granulated cytoplasm. When stained (Figs. 5 and 6), there appear two deeply stained masses in the spore, a sporoplasm which looked as a clear space in the fresh spore and the coiled polar filament as was the case in *Thelohania magna* (Kudo, 1920) or *Nosema apis* (Kudo, 1920a). The polar capsule could be detected in some spores where the polar filament was extruded (Fig. 7). The polar filament seemed to be coiled up in a way similar to *Thelohania magna*. The doubly coiled condition which was recognized in *Nosema apis,* was not observed. Fresh spores measure 4.2 to 4.7μ long by 2.7 to 3μ. The length of polar filaments extruded under the influence of mechanical pressure, fixed with sublimate alcohol and stained with Giemsa's stain, varies from 75 to 100μ.



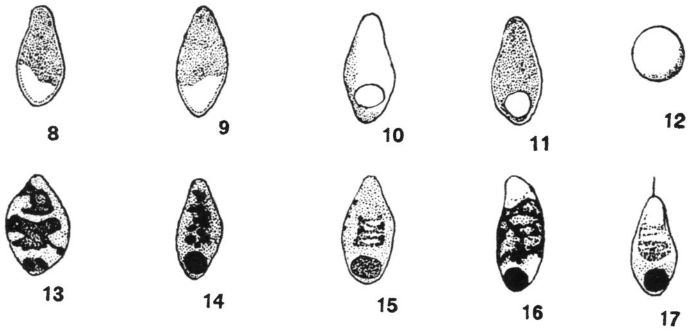
Figs. 1 to 7. Spores of *Nosema cyclopis* nov. spec. 1 to 3, fresh spores. 4, an optical cross-section. 5, a spore stained with Giemsa's stain. 6, a spore stained with Heidenhain's iron hematoxylin. 7, a spore mechanically compressed and stained with Giemsa's stain, the greater part of the polar filament is not shown. × about 2350.

Nosema infirmum nov. spec.

Habitat: In the fat body, reproductive organs and muscle of *Cyclops albidus*. New City pond and the outlet of Conger's Lake (August and September).

This species was noticed in one out of twelve host specimens obtained from New City pond on August 29, and in twenty-one out of 153 collected in the outlet of Conger's Lake on September 6. The animals collected in the same creek from which *Cyclops fuscus* infected by *Nosema cyclopis* were obtained, proved to be free from the infection. The infected animals were as strikingly whitish opaque in color as in the case already mentioned. However, they showed a marked decrease in activity compared with normal ones. While the apparently uninfected individuals were hard to capture with a pipette, the infected animals were easily caught by the same means.

The effect of the Microsporidian upon the host body in this case seems to be fatal. In the collection from the latter locality, I found fifteen dead animals completely filled with the spores and extremely whitish opaque in appearance, which condition made such animals conspicuously visible against the brownish bottom soil of the aquarium in which they were kept. As no observations were carried out, due to the lack of time at that time, on the infected animals kept in the aquarium for any length of time, it is hard to determine the cause of the death of these *Cyclops albidus*. I am, however, led to consider that the Microsporidian infection was more or less directly responsible for the death of the host animals.



Figs. 8 to 17. Spores of *Nosema infirmum* nov. spec. 8 to 11, fresh spores. 12, an optical cross-section of a fresh spore. 13 and 14, young spores, stained with Giemsa's stain. 15, spore stained with Heidenhain's iron hematoxylin. 16, a spore stained with Giemsa's stain. 17, a spore mechanically compressed and stained with Giemsa's stain, the greater part of the polar filament is not shown. × about 2350.

The spores are pyriform (Figs. 8 to 17). The anterior end is rounded at the tip. The posterior extremity is either more pointed or rounded than the anterior. The shape of the spore is however distinctly different from that of the species just described. The spore membrane is thin, but is slightly thicker than that of *Nosema cyclopis*. In cross-section, the spore is circular (Fig. 12). The broadest part passes through the middle of the long axis of the spore. The size and shape vary to a certain extent. In the fresh state, there is seen a clear space which is either oval or irregularly triangular in form, at or near the posterior extremity of the spore (Figs. 8 to 11). The rest of the contents of the spore appear finely granulated. When stained, the cytoplasm is seen to be located at the posterior end where a clear space was noticed in the fresh state. The polar filament is more distinctly visible even in unextruded condition than the former species (Figs. 15 and 16). The fresh spores measure 5.6 to 6.4μ long by 3μ broad. The length of the polar filament, as determined in the same way mentioned in the first species, varies from 90 to 115μ.

Observations upon the schizogony and sporogony are insufficient and I cannot give satisfactory full accounts of the changes that take place. As far as the observation up to the present date, is concerned, the vegetative forms of the two species, the schizonts as well as sporonts, can not be distinguished from each other unless spores are found with them, in which case, the distinction between the two species is comparatively easily done.

The youngest schizonts are rounded bodies, each with a single deeply stained chromatic mass. The body increases in size as the nucleus multiplies repeatedly, forming spherical, oblong or elongated bodies with 2, 3, 4, 5 or 6 nuclei. The ultimate products seem to be uninucleate rounded sporonts. Each sporont develops into a single spore which characterizes the genus Nosema. The development seems to be much different from *Thelohania magna* or *Thelohania illinoisensis,* but similar to that of *Nosema bombycis* (Kudo, 1916) or *Nosema baetis* (see my account).

The difference in the form, appearance and size of the spores of the two species, leads me to record them as two distinctly different species.

An examination of previous records shows that in every case the absolute proof of the microsporidian nature of the parasites, which is, above all, the presence of a polar filament in each spore, is lacking. Strictly speaking, therefore, one can not say whether any Microsporidia were previously found in copepods or not, without reexamining the preparations if such can be obtained.

The nature of "Pilzsporen" found and mentioned by Claus (1863: 87) in the body cavity of *Cyclops* species, remains undetermined, although he seemed to have seen bodies similar to the spores of *Nosema bombycis.*

Moniez (1887) described briefly the following three species, two of which were later studied by Pfeiffer (1895) and the other by Schröder (1914).

Nosema (?) *parva* Moniez

1887, *Nosema parva,* Moniez, 1887: 1313.

1895, *Glugea leydigii,* Pfeiffer, 1895: 83, 86.

Habitat: *Cyclops* spp. at Lille and Weimar.

Pfeiffer states that the species invades the fat bodies and reproductive organs.

Vegetative form: Moniez simply states that the sporogenic masses are relatively voluminous. Pfeiffer describes "cysts" with spores are rounded or elongated. The "cyst" with macrospores is about half the size of that with microspores.

Spore: After Moniez: oval, with a clear space regularly at one end. Size 3.5μ by 2μ. After Pfeiffer: pyriform, with a clear spot at rounded end. Size 8μ by 5μ.

Remarks: The identity of the two forms is very doubtful because of the unusual difference in size of spores. The description is so brief and incomplete that one cannot place the species definitely to any genus. The original nomenclature is retained.

Thelohania acuta (Moniez)

1887, *Microsporidium acuta,* Moniez, 1887: 1314.

1914, *Thelohania acuta,* Schröder, 1914: 324-327.

Habitat: *Cyclops gigas* and *Daphnia pulex* at Lille and in Germany.

Schröder states that the infection was chiefly noticed in the fat bodies.

Vegetative form: Not described.

Spore: After Moniez: The spore terminates in a sharp point, and measures 5μ long by 2μ broad. After Schröder (on fixed specimens): Elongated pyriform; one end terminates in a blunt point, while the other is rounded. Circular in cross-section. A pyriform polar capsule not longer than the half of the spore, is seen at the anterior half. The rest of the spore is filled with cytoplasm which contains a spherical vacuole at the posterior end. The polar filament could not be extruded. Size same to that measured by Moniez.

Thelohania virgula (Moniez)

1887, *Nosema virgula,* Moniez, 1887: 1313.

1895, *Glugea virgula,* Pfeiffer, 1895: 86.

Habitat: *Cyclops* spp. at Lille and Weimar.

Vegetative form: Moniez states simply that the sporogenic masses reach 30μ by 20μ.

Spore: After Moniez: pyriform, one end rounded, the other sharply pointed. Anterior part is often bent to one side. A large vacuole at the rounded end, Size 8μ by 3μ. After Pfeiffer: spores (eight in his figure) are always arranged in a stellate form. Size 8μ by 5μ.

Remarks: The identity of these two forms is again doubtful. Pfeiffer's figure suggests that the species, if it is a Microsporidian, may belong to the genus Thelohania. It is provisionally placed there.

A parasite seems to have been observed by Schmeil (1891), Schewiakoff (1893) and Pfeiffer (1895).

Glugea schmeili Pfeiffer

1891, Myxosporidia, Schmeil, 1891: 19-21.

1893, Myxosporidia, Schewiakoff, 1893: 15-25.

1895, *Glugea schmeilii,* Pfeiffer, 1895: 84-86.

Habitat: *Cyclops* sp., *Diaptomus coeruleûs* and *D. salinus* at Halle and Weimar.

Schewiakoff states that the species forms conspicuous masses in the body cavity and the spores are found "on" the muscle. Both Schmeil and Schewiakoff remarked on the opacity of the body of the infected animals.

Vegetative form: Schewiakoff studied the continuous changes in living animals using apochromatic objective 4 mm., and states as follows: The amoeboid forms are found in the body cavity of the host. They are uninucleate. The cytoplasm is finely granulated, and forms hyaline lobose pseudopodia from any part of the surface. A nucleus and a contractile vacuole are present. Size varies from 7 to 20μ long by 3 to 6μ broad. They creep around on the epithelial and muscle cells. They become encysted. Plasmotomy of two or three individuals was frequently noticed. In the cyst, the nucleus or nuclei divides into a large number. Each daughter nucleus becomes the center of a spore.

Spore: After Schewiakoff: oval, measuring 3.2 to 4μ in length. At the broader end, a homogeneous, spherical (1.6μ in diameter) and refractive nucleus is located. Each "spore" further divides into two, the nucleus undergoing mitosis (number of chromosomes eight).

Remarks: It is highly doubtful if the amoebula with a contractile vacuole, mentioned by Schewiakoff, is a stage in the life cycle of the present paratite. Assuming that this is a Microsporidian, then it should be placed in the genus Glugea, as Pfeiffer did.

The following two more species described by Fritsch (1895) who thought them to be Microsporidia, and placed them in the genus Glugea, are much more doubtful forms than the preceding species.

Glugea colorata Fritsch

1895, *Glugea colorata,* Fritsch, 1895: 80-81.

Habitat: *Diaptomus gracilis* in Austria. The parasite formed masses of olive green or burnt sienna in color.

"Cyst" contained only 5 or 6 spores.

Glugea rosea Fritsch

1895, *Glugea rosea,* Fritsch, 1895: 81.

Habitat: *Cyclops strenuus* in Austria (June). One host animal rose-colored was observed.

Spore: Two kinds: one, smaller, oval with a somewhat pointed end and yellowish in color; the other, larger and elongated pyriform with or without vacuole.

Compared with these six ambiguous species of Microsporidia (?), the two American forms described differ greatly. Hence I have designated them by new specific names. On obtaining more material, I shall try to work out the life history of the Microsporidia.

SUMMARY

1. Two new Microsporidia, *Nosema cyclopis* nov. spec., parasitic in *Cyclops fuscus,* and *Nosema infirmum* nov., parasitic in *Cyclops albidus,* are described.

2. The effect of the infection of *Nosema infirmum* upon its host body, seems to be fatal.

3. The former papers which recorded the occurrence of Microsporodia-like parasites in copepods, are reviewed.

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